Animal Science Papers and Reports vol. 40 (2022) no. 2, 171-184
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Effect of immunocastration on the steroid hormones content, serum lipid profile, and fatty acid profile in tissues of porkers fed dry or wet diet

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(Accepted May 4, 2022)

The question we were going to answer was whether the combination of immunocastration and dry or wet diet can help us deepen further the reduction of the boar taint following immunocastration. Immunocastration is an alternative to surgical castration. It has been agreed that its use heightens animal welfare, which is of full consumers' acceptance, though it is not as efficient removing the boar taint as the surgical castration. Evidence exists however, that the level of constituents causing the taint can be moulded by fed diets. The study was conducted on 450 porkers, distributed among 6 sex*diet groups. The first three groups – gilts, surgically and immunocastrated boars – received complete compound dry ration, while the other three were fed fermented liquid diet of similar nutritional value to that offered in the dry diet. The immunocastrated porkers received two doses of synthetic gonadoliberin Improvac®. Cholesterol parameters, 17β-estradiol, testosterone, and androstenone were determined in plasma. Total lipids for the fatty acid analysis and skatole contents were assayed in the *longissimus* muscle fat, backfat, and perirenal fat. The administration

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of synthetic gonadoliberin resulted in a substantial decrease of the plasma androgen level compared to that observed in the surgically castrated porkers. This effectively prevented the appearance of a higher concentration of skatole in the tissues. Liquid diet also helped to level off the unfavourable differences regarding boar taint present in dry feeding between surgically castrated and immunocastrated barrows.

KEYWORDS: boar / castration / diet / skatole / swine / taint

The unpleasant odour, referred to as "boar taint", that may accompany the meat of boars, especially those that have reached full sexual maturity, can be a deterrent factor in pork marketing. This is due to the higher content of skatole (3-methylindole) and sex hormones (in particular androgens) in the flesh of intact males [Aldal *et al*. 2005]. Factors affecting the levels of androstenone and skatole in fat, and thus the occurrence of boar taint, are varied. For the androstenone, the key factors are genetics and sexual development stage, while the level of skatole is mainly affected by the feed composition and rearing conditions [Aldal *et al.* 2005]. One way to reduce the boar taint is to slaughter the pigs before they reach sexual maturity [e.g., Zamaratskaia *et al*. 2004]. Yet, studies have shown that this defect cannot be completely eliminated in this manner [Aldal *et al*. 2005]. An additional drawback of fattening of uncastrated male pigs is their aggressiveness [Fredriksen *et al*. 2011]. High level of aggression and frequent fighting handicap welfare and growth performance of the pigs [Zamaratskaia *et al*. 2008]. As the surgical method of castration, considered inhumane, faces growing opposition of consumers while slaughter of sexually immature animals is unprofitable, increased usage of stimulation of the formation of specific antibodies against synthetic gonadoliberin (GnRH), administered in the form of a vaccine is observed [Yuan *et al*. 2012, Moore *et al*. 2017]. Synthetic GnRH is recognized by the organism as "alien" so the organism produces antibodies against it, which later can bind to natural GnRH and inhibit its action [e.g., Grela *et al*. 2013] – its use improves animal welfare (painless, surgery-free, no frustration related to restraint), what is accepted by consumers and animal rights activists.

The almost immediate loss of testicular function following the surgical castration results in a significant reduction of the biosynthesis and secretion of testosterone and androstenone. Reduced secretion of the sex hormones may slow down lipid catabolism and indirectly affect the quantity and quality of pork fat [Škrlep *et al*. 2010]. It has been proven – e.g., Aluwé *et al*. [2015] – that individuals subjected to surgical castration have higher fat content in comparison to gilts, and especially in comparison to intact boars. It can therefore be assumed that immunological castration can also affect lipid metabolism and fatness of the carcass.

In modern pig feeding, the diets should positively affect health of the animals, ensure possibly highest utilization of nutrients, and reduce feeding costs. From the consumer point of view pork, as the final product, should also possess health-promoting qualities. An example of such an approach is liquid feeding with fermented feed using by-products from the agri-food industry [Grela *et al*. 2018]. In fact, liquid feeds are recommended by the Health and Food Safety Directorate-General of the EU

as a mean of reducing competition at feeding. The potential of fermented liquid feed, as an alternative to the use of growth-promoting antibiotics, has been discussed in some recent reviews [e.g., Missotten *et al*. 2015]. What is more, regardless the feeding system, dry or liquid, the diet composition may support usefulness of immunocastration by affecting the skatole levels, what may be concluded from the reports of Pauly *et al*. [2008], Zamaratskaia and Squires [2009], and Weiler *et al*. [2013].

The aim of the study was to determine the effect of immunocastration on the content of steroid hormones, cholesterol and triacylglycerols in the blood and on the fatty acid profile of the *longissimus lumborum* (MLL) muscle fat, backfat (BF) and perirenal fat (PF) of pigs fed dry or wet diet of similar nutritional value.

Material and methods

The experimental procedures used throughout this study were approved by the II Local Ethics Committee on Animal Experimentation of University of Life Sciences in Lublin, Poland, (Resolution No. 35/2011 of 07 June 2011).

Animal housing and feeding

The study was conducted on 450 pigs of the PIC crossbred line, distributed among 6 groups of 75 and housed in pens of 15 pigs each. The first three groups, distributed according to sex – i.e. gilts (G), surgically castrated barrows (BC), and immunocastrated barrows (BI), received complete compound dry rations, while the other three, distributed in the same manner according to sex, were fed fermented liquid diet. The Gs serve in this investigation as the reference group since their final raw product – pork – is accepted by the consumers as it is. The exact composition of the diets was published in the paper of Grela *et al*. [2018], in which we compared the effect of employing different diets (dry vs. wet) on growth and feed conversion of the sex groups. Hence, for the sake of the present analysis we present only the nutritive values of the diets, in Table 1. The BCs were castrated at the age of 5 days, while the BIs received two 2-ml doses of synthetic gonadoliberin (Improvac®, Pfizer Ltd.), containing 200 μg of GnRH/ml, at the age of 74 and 141 days. The fattening period was divided into two stages: GROWER (30-70 kg live weight) and FINISHER (71-115 kg LW). The dry feed (DRY) was provided *ad libitum* with free access to drinking water, and the liquid feed (WET) was offered 3 times a day.

Experimental activities

The pigs were weighed at 70 days (start), 130 (grower) and 168 (finisher) days of age. Blood samples were collected after 20 and 80 days of the experiment.

Feed analysis

The contents of the basic nutrients (crude protein, crude fibre and ether extract), amino acids, acid detergent fibre (ADF), neutral detergent fibre (NDF) and minerals

(Ca, P, Na) in the feeds were determined according to the AOAC procedures [AOAC, 2012].

Blood collection and analysis

Blood samples were collected twice from 60 tagged animals, 10 from each group (2 per pen), at a body weight of about 40-45 kg and 90-95 kg. The animals had no access to feed for 12 hours prior to blood collection. Blood samples were taken from pigs of intermediate body weights within a pen by a veterinarian, from the jugular vein into 10-ml heparinized tubes.

Tests from Cormey were used for spectrophotometric (Cary 300 Research-Grade UV-VIS Spectrophotometer, Agilent Technologies, USA) determination of the content of selected lipid metabolism indices in the plasma, i.e. total cholesterol (CHOL), the HDL cholesterol, and triacylglycerols (TG). The low-density lipoprotein (LDL) cholesterol fraction was calculated according to the formula proposed by Friedewald *et al*. [1972]:

$$\text{LDL (mmol l}^{-1}) = \text{total cholesterol} - \text{HDL} - \text{triacylglycerols}/2.2$$

Contents of plasma 17β-estradiol (pg ml^{-1}), testosterone (ng ml^{-1}) and androstenone (ng ml^{-1}) were determined. The hormones were extracted from the plasma with dichloromethane and then analysed by high-performance liquid chromatography (HPLC, Beckman, Gold System, USA). The analysis was carried out using a LiChrospher 100 reversed phase column (250 x 4 mm inner diameter, 5 μm; Merck, Germany). The mobile phase, consisting of 0.25% orthophosphoric acid and acetonitrile, was pumped at a rate of 0.8 ml/min (125 SM; Beckman). Hormones were separated in an acetonitrile gradient (40-100% in 20 min), followed by UV detection at 220 nm (DAD 168; Beckman). Dehydrocholic acid was used as the internal standard.

Tissue collection and analysis

After 98 experimental days and body weight averaging 115 kg, all pigs were shipped to an abattoir, following a 12h fast.

Samples of MLL, perirenal fat and backfat were collected 24 h after slaughter from 10 animals of each group. The tissues were sampled from carcasses of 2 pigs of an average body weight within a pen. The MLL samples were taken from near the last thoracic and first lumbar vertebra region. Backfat was sampled from above the scapula by cutting out a slab about 6 cm wide and 20 cm long from the forequarter. The perirenal fat samples were collected from the right semi-carcass. Immediately after collection, the fat tissues were stored individually in plastic bags at about -20°C. Total lipids for the fatty acid analysis were extracted from the backfat, perirenal fat, and MLL muscle fat with a chloroform/methanol mixture, according to Folch *et al*. [1957].

Assayed were the levels of the following fatty acids: 12:0; 14:0; 16:0; 16:1, n-7; 16:1, n-9; 17:0; 17:1, n-10; 18:0; 18:1, n-9; 18:1, n-7; 18:2, n-6; 18:3, n-3; 20:0; 20:1, n-11; 20:2; 20:4, n-6 which we grouped into saturated fatty acids (SFA), mono- and polyunsaturated fatty acids (MUFA. PUFA), for the sake of the statistical analysis.

The percentages of fatty acid methyl esters were estimated by gas chromatography on a Varian CP-3800 chromatograph. The operating conditions for fatty acid separation were: CP WAX 52CB DF 0.25 mm capillary column 60 m in length, gas carrier – helium, flow rate 1.4 ml/min, column temperature 120°C gradually increasing by 2°C/ min up to 210°C, determination time 127 min, feeder temperature 160°C, detector temperature 160°C, other gases – hydrogen and oxygen. The atherogenicity (AI) and thrombogenicity (TI) indices were calculated according to formulas proposed by Ulbricht and Southgate [1991]. The hypocholesterolaemic/ Hypercholesterolaemic ratio (h/H) was determined according to Fernández *et al*. [2007].

Skatole content in the fat was determined using a spectrophotometric method based on the Chernoff's modification of Ehrlich's indole reaction with 4-dimethylaminbenzaldehyde [Mortensen and Sørensen, 1984].

Statistical analysis

Based on the introductory ANOVA runs' results, the final fixed model accounted for:

$$y_{ijkl} = D_i + S_j + F_k + (D \times S)_{ij} + e_{ijkl}$$

where:

D_i – denotes the diet (DRY, WET);

S_j – sex group (G, BC, BI);

F_k – fattening stage (Grower, Finisher);

$(D \times S)_{ij}$ – interaction between diet and sex effects;

e_{ijkl} – the residual.

The ANOVA model for the fatty acids (n = 10) and skatole levels (n = 10) omitted the fattening stage. Appropriate pre-planned orthogonal contrasts were performed for all the factors to evaluate the significance of the differences between the levels of the effects. The *p*-values were subjected to Bonferroni correction to account for multiple comparisons.

Results and discussion

The diets (Tab. 1)

The study was set up at a commercial pig farm of which the management is vitally interested in providing the animals the highest level of welfare possible. That is why the liquid feeding system emerged upon designing the investigation, besides immunocastration. Due to the on-farm limitations we could not compose identical DRY and WET diets to unbiasedly compare the effects of the feeding systems. Nonetheless, we managed to obtain diets of comparable nutritional values. Slight differences were only found in the content of detergent fibre fraction (NDF and ADF)

Table 1. Nutritive value of growing (30-70 kg) and finishing (71-115 kg) DRY and WET diets per 1 kg feed DM (excerpt from Grela *et al*. [2018])

Diet	DRY		WET	
Fattening period	30-70 kg	71-115 kg	30-70 kg	71-115 kg
Metabolizable energy[1] (MJ)	13.25	13.11	13.21	13.11
NDF[2] (g)	256	278	221	228
ADF[3] (g)	84	93	72	73
Crude protein (g)	169	156	173	159
Lysine (g)	10	9	10	9
Methionine+cysteine (g)	6	6	6	6
Threonine (g)	7.02	6.48	7.13	6.51
Tryptophan (g)	2	1.85	2.10	1.91
Calcium (g)	8	7	8	8
Total phosphorus (g)	5	5	5	5
Sodium (g)	2	2	2	2
Ether extract (g)	31	30	24	23
Palmitic acid (g)	6	6	4	4
Stearic acid (g)	2	2	1	1
Oleic acid (g)	10	10	6	6
Linoleic acid (g)	9	9	10	10

[1]Metabolizable energy was calculated according to Kirchgessner and Roth [1983].
[2]NDF – neutral detergent fibre.
[3]ADF – acid detergent fibre.

and in the content of fat and carbon 18 fatty acids. The WET diet proved beneficial from the body gain and feed conversion ratio points of view [Grela *et al*. 2018].

Cholesterol breakdown, triacylglycerols, and steroid hormones contents (Tab. 2)

The Grower stage. The Grower stage is not directly involved in moulding the quality of pork as a raw swine product. Moreover, the surgically castrated boars entered the experiment with fully stopped activity of the testicles while the process of immunocastration commenced more or less at the same time as the investigation, with the second dose at around 50 kg of live weight. Hence, we shall only briefly present the experimental factors' effects upon the studied features, at this stage.

When the cholesterol and the triacylglycerols are concerned, only the HDL level appeared to be somewhat affected by the diet, being higher in WET ($p = 0.077$) and by sex, reaching lowest values in Gs ($p = 0.063$). As a consequence, the proportion of HDL significantly increased in WET ($p = 0.028$) while it was highest in BIs (sex group differences significant at $p = 0.089$). Apparently, the applied WET diet, and to a lesser extend immunocastration, helped the organism get rid of surplus cholesterol, at this stage. Whereas this aspect of the cholesterol metabolism is less important in the terminal animals, the breeding ones can benefit from employing the wet feeding.

The situation with the hormone levels is a bit entangled. The diets affected the contents of all the three hormones. 17β-estradiol was lowered in WET ($p = 0.064$) due to lower values than in DRY in the castrates. In particular, the results of BIs, significantly higher than in any other diet-sex group, dropped by some 6 peg ml^{-1}, compared to the

Table 2. Least-squares means for cholesterol breakdown, triacylglycerols, and steroid hormones contents in the plasma of porkers[1] across diet, sex group, and fattening stage

Item	Fattening stage[4]	Diet/sex group[2]						*p*-values[3]	
		DRY			WET			D	S
		G	BC	BI	G	BC	BI		
CHOL	Grower	1.98	2.34	2.42	2.39	2.44	2.05	0.621	0.180
(mmol l^{-1})	Finisher	1.68^{b}	2.02^{a}	2.08^{a}	1.75^{b}	1.49^{b}	1.62^{b}	0.001	0.320
HDL	Grower	1.14^{b}	1.42^{a}	1.47^{a}	1.41^{a}	1.53^{a}	1.40^{a}	0.077	0.063
(mmol l^{-1})	Finisher	0.88^{b}	1.05	1.14^{a}	1.12^{a}	0.89^{b}	1.04	0.844	0.132
LDL	Grower	0.70	0.79	0.77	0.69	0.70	0.67	0.162	0.925
(mmol l^{-1})	Finisher	0.74	0.72	0.76	0.68	0.67	0.69	0.180	0.831
TG	Grower	0.33	0.28	0.26	0.25	0.28	0.22	0.728	0.961
(mmol l^{-1})	Finisher	0.31^{a}	0.18^{b}	0.18^{b}	0.18^{b}	0.16^{b}	0.13^{b}	0.036	0.056
%HDL	Grower	57.39^{b}	60.81	59.04^{b}	59.31	63.02	68.58^{a}	0.028	0.089
	Finisher	53.24^{b}	51.84^{b}	54.76^{b}	64.33^{a}	59.77^{a}	63.96^{a}	<0.001	0.299
17βestradiol	Grower	18.82^{b}	19.41^{b}	28.10^{a}	20.39^{b}	17.92^{b}	29.81^{a}	0.064	0.015
(pg ml^{-1})	Finisher	20.89^{bc}	18.77^{c}	27.80^{ab}	24.39^{b}	21.25^{b}	32.79^{a}	0.031	0.011
Testosterone	Grower	2.74^{c}	2.89^{bc}	4.25^{a}	3.49^{b}	3.21^{b}	4.89^{a}	0.001	0.002
(ng ml^{-1})	Finisher	1.37^{b}	1.49	1.48	1.49	1.62^{a}	1.57^{a}	0.041	0.005
Androstenone	Grower	1.02^{a}	1.23^{a}	1.15^{a}	0.59^{b}	0.64^{b}	1.08^{a}	0.035	0.013
(ng ml^{-1})	Finisher	0.61^{a}	0.65^{a}	0.67^{a}	0.41^{b}	0.47^{b}	0.55^{ab}	<0.001	0.021

[1]G – gilts; BC – surgically castrated barrows; BI – immunocastrated barrows.
[2]Within a row: differences between means bearing different superscripts significant at $p \leq 0.05$.
[3]D (diet) – DRY vs. WET; S (sex group) – G vs. BC vs. B.
[4]Grower (30-70 kg) and Finisher (71-115 kg).

DRY diet. With the results for the Gs remaining at the same level across the diets the difference of the sex group effects proved to be significant at $p = 0.015$.

The testosterone levels were consistently lower in each sex group in DRY ($p = 0.001$). The values for the BIs within a diet exceeded significantly ($p \leq 0.05$) those for Gs and BCs, what largely contributed to the overall significance of the sex group differences ($p = 0.002$).

Opposite to the testosterone levels the androstenone ones were lower ($p = 0.035$) in WET, but significant drop regarded only the Gs and BCs, resulting in the overall sex group differentiation significant at $p = 0.013$.

Discussing the boar taint issue, the testosterone levels were comparable between Gs and BCs within DRY and within WET while the significance of the higher testosterone level in BIs was not affected by the diet. On the other hand, the androstenone content was successfully lowered by WET, although that regarded only Gs and BCs.

Administration of the first dose of the synthetic gonadoliberin (Improvac®) vaccine at 74 days of age did not affect testicular function, as indicated by the significantly higher β-estradiol and testosterone levels in BIs. According to Lealiifano *et al*. [2011], such a dose of the vaccine only initiates the immune response. Research by Kopera *et al*. [2008], however, indicated a decrease in the plasma level of androgens after the first injection of the preparation, which may have been caused by the use of more aggressive adjuvants, e.g., DEAE-dextran, thiomersal or urea.

The Finisher stage. This is the stage at which the quality of raw pork is finally shaped, both in terms of its health-promoting and boar taint properties.

The WET diet proved not only to lower the content of the overall cholesterol ($p = 0.001$), but also levelled off the, present in DRY, significant cholesterol level difference between Gs and castrates (Gs < castrates, $p \leq 0.05$). Another beneficial effect of WET can be noted in %HDL. Regardless the lack of the diet and sex group influences on the absolute contents of HDL and LDL, %HDL was significantly ($p < 0.001$) heightened in WET, more or less equally for each of the sex groups.

Whereas cholesterol had been known as the stroke risk factor for long a time, the triacylglycerols were identified as independent stroke risk factors only at the turn of the centuries [e.g., Tanne *et al*. 2001]. In the present investigation we found the TG content lower in WET ($p = 0.036$), though the significant DRY to WET drop was recorded only in Gs ($p \leq 0.05$), making the difference between sexes significant at $p = 0.056$.

Composing adequate diets to help control CHOL and TG levels (at least), while increasing the beneficial HDL ratio in the raw pork can be successfully implemented in the swine production practice.

Talking the issue of the boar taint, largely dependent on the level of steroid hormones, the inclusion of estradiol has a reason. Estradiol, often referred to as the "female hormone", is a member of the estragen trio (estradiol, estriol, estrone), and is the most androgenic of the three [e.g., Stillwell 2016]. Indeed, plasma estradiol was suggested as a predictor of androstenone levels by Punier *et al*. [2016] and the correlation between their levels was estimated at above 0.8 [Dugué *et al.* 2020].

In the present study, both sex group and diet appeared to notably ($p = 0.041$ down to $p < 0.001$) affect the levels of the hormones. 17β-estradiol contents were heightened by WET ($p = 0.031$) in all the sex groups. BIs were the group exhibiting highest levels of estradiol in both DRY and WET, while BCs the lowest, differing significantly from BIs in DRY, and from Gs and BIs in WET ($p \leq 0.05$). The testosterone levels were also increased in WET ($p = 0.041$), mostly due to the increase in BCs and BIs, making the sex group differences significant at $p = 0.005$. Yet, the lack of a significant difference between BCs and BIs in DRY still remained in WET. The androstenone contents in the serum were the only to significantly ($p < 0.001$) drop in WET. Within the diets the sex groups did not statistically differ. From the boar taint point of view the WET diet helped to substantially decrease the androstenone contents but not the estradiol and testosterone ones, nor did it manage to compensate for the significant difference of 17β-estradiol contents between BCs and BIs, which was already present in DRY.

Evidently, the second dose of the synthetic gonadoliberin (Improvac®) vaccine, administered towards the end of the Grower stage, resulted in the expected anabolic effect of the sex hormones. This effect is the result of the high antibody titre attained in order to ensure the effect of castration when the boars reach sexual maturity [Claus *et al*. 2007]. According to Claus *et al*. [2007], a gradual decrease in testosterone concentration (only after the second dose) is not due to a low level of antibody synthesis, but to the gradual passage of androgens stored in adipose tissue into the

blood. Androgens stored in the adipose tissue are released into the blood very slowly. Claus *et al*. [2007] reported also that after the second vaccination anti-GnRH antibody titres rapidly increased to the maximum level, resulting in a complete blockade of GnRH secretion and inhibition of steroidogenesis in the Leydig cells after just three days [Brunius *et al*. 2011]. As a consequence, the testes stop functioning completely, and the odorous substances already present are eliminated from the body between vaccination and slaughter. A significant reduction in testosterone secretion after the second dose of the vaccine was reported by Albrecht *et al*. [2012].

The higher levels of testosterone in BCs and BIs were associated with an increase in HDL cholesterol in the plasma, which probably resulted from hydrolysis of triacylglycerols and phospholipids contained in HDL, stimulated by hepatic lipase. Hepatic lipase activity is stimulated by androgens and has such an effect on all lipoproteins containing TG. Therefore, a decreased androgen level may indirectly cause an increase in plasma triacylglycerols [Jansen *et al*. 2002], as observed in the gilts, especially those fed DRY. Increased TG content may result in health consequences and reduce the nutritional value of pork [Bee *et al*. 2002].

Skatole contents (Tab. 3)

This aspect of the research is related to the intensity of the boar taint in pork. Both the feeding system and the sex group influenced ($p \approx 0.04$) the level of skatole in the fats of interest – WET proved to lower the skatole contents across sex groups and eliminated the significant difference between BIs and Gs in the MLL fat and backfat, present in DRY. No significant differences were found between BCs and BIs. Hence, we can generally conclude that skatole levels can be efficiently controlled by the feeding system whereas sex differences are only those between Gs and castrates.

Table 3. Least-squares means for skatole contents in the *longissimus* muscle fat, backfat, and perirenal fat of porkers[1] across diets and sex groups

Tissue	Diet/Sex group[2]						*p*-values[3]	
	DRY			WET			D	S
	G	BC	BI	G	BC	BI		
Musculus longissimus lumborum (µg/mg)	0.23[b]	0.25	0.28[a]	0.21[b]	0.24	0.25	0.035	0.041
Backfat (ppm)	0.07[b]	0.08	0.08[a]	0.06[b]	0.07	0.08	0.045	0.043
Perirenal fat (ppm)	0.08	0.08	0.09[a]	0.06[b]	0.07	0.08[a]	0.044	0.043

[1]G – gilts; BC – surgically castrated barrows; BI – immunocastrated barrows.
[2]Within a row: differences between means bearing different superscripts significant at $p \leq 0.05$.
[3]D (diet) – DRY vs. WET; S (sex group) – G vs. BC vs. B.

As Zammerini *et al*. [2012] reported, diet can significantly affect the concentration of skatole in the pig fat. Skatole is formed from the amino acid L-tryptophan during the degradation of proteins. It is produced by bacteria commonly found in the large intestine of monogastric animals and is responsible for giving the meat a faecal-like odour. Skatole concentration depends on genetics, age, sex, diet and breeding [Zamaratskaia

et al. 2004]. An *ad libitum* feeding regime increases skatole deposition in the adipose tissue as compared to a restricted feeding regime, while free access to water (and/or wet feed) leads to lower skatole levels present in the dry feeding system.

Our research indicates that a diet of fermented liquid feed not only reduces skatole concentrations, but also aldosterone levels in the blood. According to Aldal *et al*. [2005], this reaction could be linked to the effect of liquid feed on regulation of the maturation process, while the supply of large amounts of water and lactic acid bacteria with fermented liquid feed can modify bacterial activity in the gut and thereby alter the rate of skatole synthesis. In addition, the intestinal transit time affects skatole absorption through the intestinal walls. The reduction can also be explained by the inhibition of apoptosis in the intestine, resulting in a decrease in the amount of tryptophan and its availability for skatole production. Furthermore, although androstenone and skatole have different origins, elevated levels of androstenone are accompanied by elevated levels of skatole, with a correlation coefficient of about 0.3 [Škrlep *et al*. 2010]. Both substances are lipophilic (they accumulate in the adipose tissue of pigs). Their metabolic pathways seem to be interdependent, and according to Škrlep *et al*. [2010] androstenone, together with other testicular steroid hormones, affects skatole metabolism in the liver.

Grouped fatty acid contents in the tissues' fat (Tab. 4)

Of the fatty acids profile the overall characteristics: MUFA, PUFA, and the h/H ratio are associated with heightened health-promoting properties of animal products, whereas increased SFA, AI, TI, and the n-6/n-3 ratio values are undesirable from the consumer's wholesomeness point of view. Ulbricht and Southgate [1991] considered the AI and TI indexes to be better indicators of atherogenicity and thrombogenicity than the PUFA/SFA ratio. This is because not all SFA acids are hypercholesterolaemic, and in addition to PUFA, MUFA also exhibit a protective effect.

As the table shows, the undesirable parameters of fatty acid composition were decreased by WET in MLL ($p \approx 0.03$ to 0.23) and in PF ($p \approx 0.04$ to 0.13) – the desirable ones were automatically increased at $p \approx 0.02$ to 0.04 for MLL and PF. It was only BF where the tendency was opposite, making the differences between diets significant at $p \approx 0.02$ to 0.04 for both the disadvantageous and advantageous fatty acid profile parameters.

While sex (gilts, barrows and boars treated with Improvac®) did not have a significant effect on the fatty acid profile in the tissues, the feeding system did, as indicated by the significant dependence between these two factors. The higher MUFA and PUFA content in the liquid-fed animals may be associated with the significant proportion of maize in the diet [Capraro *et al*. 2017]. According to Argemí-Armengol *et al.* [2021], the content of fatty acids in the body, mainly in the muscles and storage fat, is dependent on the type of feeds used, mainly the fat contained in them, and the feeding system. Too high a level of these acids has a negative effect on the oxidative stability and storage properties of meat and backfat [Wood *et al.* 2003]. Pig feeding

Table 4. Least-squares means for fatty acid collective compositions (%) and chosen fatty acid indices in the *longissimus* muscle fat, backfat, and perirenal fat of porkers[1]

Tissue	Item	Diet/Sex group[2]						*p*-values[3]	
		DRY			WET			D	S
		G	BC	BI	G	BC	BI		
	SFA	41.15^{a}	41.32^{a}	40.09^{b}	39.60^{bc}	40.03^{b}	39.14^{c}	0.034	0.057
	MUFA	51.28^{b}	51.06^{b}	51.73	52.18^{a}	51.81	52.39^{a}	0.042	0.059
Musculus	PUFA	7.10^{bc}	6.87^{c}	7.39^{b}	7.52	7.41	7.91^{a}	0.023	0.041
longissimus	AI	0.53	0.54	0.52	0.51	0.52	0.50	0.086	0.225
lumborum	TI	1.23^{a}	1.26^{a}	1.19	1.16^{b}	1.18	1.12^{b}	0.032	0.064
	h/H	2.01^{b}	1.98^{b}	2.06	2.10^{a}	2.06	2.11^{a}	0.038	0.045
	n-6/n-3	3.77	3.98	3.89	3.76	3.97	3.71	0.232	0.045
	SFA	35.72^{b}	34.70^{b}	38.11	41.78^{a}	40.27^{a}	38.33	0.044	0.277
	MUFA	50.96^{a}	52.83^{a}	47.81	42.83^{b}	43.30^{b}	46.84	0.026	0.549
	PUFA	13.04^{b}	12.31^{b}	13.93^{b}	15.24^{a}	16.09^{a}	14.63	0.037	0.471
Backfat	AI	0.43^{b}	0.40^{b}	0.47	0.51^{a}	0.51^{a}	0.45	0.041	0.136
	TI	0.98^{bc}	0.93^{c}	1.08^{b}	1.22^{a}	1.15	1.05^{b}	0.032	0.043
	h/H	2.58	2.80^{a}	2.40^{b}	2.13^{c}	2.10^{c}	2.41^{b}	0.021	0.022
	n-6/n-3	7.07^{a}	6.78^{b}	7.29^{a}	6.62^{b}	6.76^{b}	6.51^{b}	0.015	0.034
	SFA	48.15^{b}	49.90^{a}	47.66^{bc}	46.67^{c}	48.20^{b}	46.31^{c}	0.041	0.037
	MUFA	43.66	42.26	43.45	43.71	42.78	43.70	0.042	0.059
	PUFA	7.63^{bc}	7.24^{c}	8.33^{b}	8.99	8.33^{b}	9.33^{a}	0.026	0.042
Perirenal fat	AI	0.68	0.73	0.66	0.64	0.69	0.64	0.132	0.145
	TI	1.69	1.81^{a}	1.64	1.56^{b}	1.67	1.53^{b}	0.037	0.061
	h/H	1.60	1.50^{b}	1.66^{a}	1.70^{a}	1.58	1.68^{a}	0.044	0.068
	n-6/n-3	6.95^{a}	6.87^{a}	6.64	6.08^{b}	6.44	6.02^{b}	0.043	0.084

[1]G – gilts; BC – surgically castrated barrows; BI – immunocastrated barrows.
[2]Within a row: differences between means bearing different superscripts significant at $p \leq 0.05$.
[3]D (diet) – DRY vs. WET; S (sex group) – G vs. BC vs. B.

in the liquid system also improved the n-6/n-3 fatty acid ratio, which is beneficial in terms of human nutrition. The lipid quality indices h/H, AI and TI also varied somewhat depending on the sex of the pigs and the feeding system. The increased h/H ratio in the fat of the *longissimus* muscle and the perirenal fat should be considered beneficial. Reverse relationships were observed in the backfat. When the sex groups are considered, best fatty acid composition was consistently found in BIs (sex group differences significant at $p \approx 0.04$ to 0.08) in the MLL and PF, although the differences between sex groups for AI were all-over nonsignificant.

Conclusions

The conclusions are drawn based on comparison of only two diets composed to be possibly equivalent with regard to their nutritional value, yet of different form, rather than to target single issues of pork quality.

All the studied characteristics, tightly linked with the boar taint issue or with the health-promoting properties of pork can be successfully moulded by employing liquid feeding. In particular liquid feeding lowered the levels of androstenone and skatole and improved fatty acid composition in *musculus longissimus lumborum*, backfat and perirenal fat.

Combination of liquid feeding and immunocastration can be recommended in swine production practice to heighten hog wellbeing while yielding high quality pork of health promoting properties.

Conflict of interest. We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons satisfying the criteria of authorship, but having not been listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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